

## Overview

### Useful For

As an adjunct to measurement of 17-hydroxyprogesterone, androstenedione, and cortisol in the diagnosis of difficult cases of suspected 21-hydroxylase (CYP21A2) deficiency

Identifying heterozygote CYP21A2 deficiency carriers

As an adjunct to measurements of 17-hydroxyprogesterone, androstenedione, testosterone, and, in female patients, estradiol in the follow-up of children with CYP21A2 deficiency

### Special Instructions

- [Steroid Pathways](#)

### Method Name

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

### NY State Available

Yes

## Specimen

### Specimen Type

Serum

### Specimen Required

**Supplies:** Sarstedt Aliquot Tube, 5 mL (T914)

**Collection Container/Tube:**

**Preferred:** Red top

**Acceptable:** Serum gel

**Submission Container/Tube:** Plastic vial

**Specimen Volume:** 0.5 mL

**Collection Instructions:**

1. Morning (8 a.m.) specimen is preferred.
2. Centrifuge and aliquot serum into a plastic vial.

### Specimen Minimum Volume

0.4 mL

### Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	OK

### Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Ambient	14 days	
	Refrigerated (preferred)	21 days	
	Frozen	21 days	

### Clinical & Interpretive

#### Clinical Information

The adrenal glands, ovaries, testes, and placenta produce steroid hormones, which can be subdivided into 3 major groups: mineralocorticoids, glucocorticoids, and sex steroids. Synthesis proceeds from cholesterol along 3 parallel pathways, corresponding to these 3 major groups of steroids, through successive side-chain cleavage and hydroxylation reactions. At various levels of each pathway, intermediate products can move into the respective adjacent pathways via additional, enzymatically-catalyzed reactions (see [Steroid Pathways](#)).

21-Deoxycortisol is an intermediate steroid in the glucocorticoid pathway. While the main substrate flow in glucocorticoid synthesis proceeds from 17-hydroxyprogesterone via 21-hydroxylation to 11-deoxycortisol and then, ultimately, to cortisol, a small proportion of 17-hydroxyprogesterone is also hydroxylated at carbon number 11 by 11-beta-hydroxylase 1 (CYP11B1), yielding 21-deoxycortisol. This in turn can also serve as a substrate for 21-hydroxylase (CYP21A2), resulting in formation of cortisol.

The major diagnostic utility of measurements of steroid synthesis intermediates lies in the diagnosis of disorders of steroid synthesis, particularly congenital adrenal hyperplasia (CAH). All types of CAH are associated with cortisol deficiency except for CYP11B2 deficiency and isolated impairments of the 17-lyase activity of CYP17A1 (this enzyme also has 17-alpha-hydroxylase activity). In case of severe illness or trauma, CAH predisposes patients to poor recovery or death. Patients with the most common form of CAH (21-hydroxylase deficiency, which accounts for >90% of cases), the third most common form of CAH (3-beta-steroid dehydrogenase deficiency, which accounts for <3% of cases), or the extremely rare StAR (steroidogenic acute regulatory protein) or 20,22 desmolase deficiencies might also suffer mineralocorticoid deficiency, as the enzyme blocks in these disorders are proximal to potent mineral corticoids. These patients might suffer salt-wasting crises in infancy. By contrast, patients with the second most common form of CAH (11-hydroxylase deficiency, which accounts for <5% of cases) are normotensive or hypertensive, as the block affects either CYP11B1 or CYP11B2, but rarely both, thus ensuring that at least corticosterone is still produced.

In addition, patients with all forms of CAH might suffer the effects of substrate accumulation proximal to the enzyme block. In the 3 most common forms of CAH, the accumulating precursors spill over into the sex steroid pathway, resulting in virilization of female patients or, in milder cases, in hirsutism, polycystic ovarian syndrome, or infertility, as well as in possible premature adrenarche and pubarche in both sexes.

Measurement of the various precursors of mature mineralocorticoids and glucocorticoids, in concert with the determination of sex steroid concentrations, allows diagnosis of CAH and its precise type and serves as an aid in monitoring steroid replacement therapy and other therapeutic interventions.

Measurement of 21-deoxycortisol can supplement or confirm 17-hydroxyprogesterone and androstenedione measurements in the diagnosis of difficult cases of CAH presumed to be due to CYP21A2 deficiency. 11-Hydroxylation remains intact in such patients. However, since the CYP21A2 enzyme block prevents formation of 11-deoxycortisol while simultaneously increasing the concentrations of the precursor, 17-hydroxyprogesterone, unoccupied CYP11B1 starts to hydroxylate the abundant 17-hydroxyprogesterone substrate into 21-deoxycortisol. The 21-deoxycortisol accumulates, as the diminished or absent CYP21A2 activity slows or prevents its conversion into cortisol.

For other forms of CAH, the following tests may be relevant:

21-Hydroxylase deficiency:

- OHPG / 17-Hydroxyprogesterone, Serum
- ANST / Androstenedione, Serum
- 21DOC / 21-Deoxycortisol, Serum

11-Hydroxylase deficiency:

- DOCS / 11-Deoxycorticosterone, Serum
- CORTC / Corticosterone, Serum
- PRA / Renin Activity, Plasma
- ALDS / Aldosterone, Serum

3-Beta-steroid-dehydrogenase deficiency:

- 17PRN / Pregnenolone and 17-Hydroxypregnenolone, Serum

17-Hydroxylase deficiency or 17-lyase deficiency (CYP17A1 has both activities):

- 17PRN / Pregnenolone and 17-Hydroxypregnenolone, Serum
- PGSN / Progesterone, Serum
- OHPG / 17-Hydroxyprogesterone, Serum
- DHEA\_ / Dehydroepiandrosterone (DHEA), Serum
- ANST / Androstenedione, Serum

Cortisol should be measured in all cases of suspected CAH.

It has been suggested that in the pubertal patient with 21-hydroxylase deficiency, 21-deoxycortisol may be useful and better than 17-hydroxyprogesterone for therapeutic decisions.

### Reference Values

<5.0 ng/dL

Reference values apply to all ages.

### Interpretation

In untreated 21-hydroxylase (CYP21A2) deficiency, 21-deoxycortisol serum concentrations on average exceed the upper limit of the reference range 30-fold to 40-fold.

21-Hydroxycortisol measurements are particularly useful in equivocal cases of suspected 21-hydroxylase deficiency. Most untreated patients with 21-hydroxylase deficiency have serum 17-hydroxyprogesterone concentrations well in excess of 1000 ng/dL. For the few patients with levels in the range of greater than 630 ng/dL (upper limit of reference range for newborns) to 2000 ng/dL or 3000 ng/dL, it might be prudent to consider 11-hydroxylase deficiency as an alternative diagnosis. This is particularly true if serum androstenedione concentrations are also only mildly-to-modestly elevated and if the phenotype is not salt wasting but either simple virilizing (female) or normal (female or male). 11-Hydroxylase deficiency, particularly if it affects 11 beta-hydroxylase 1 (CYP11B1), can be associated with modest elevations in serum 17-hydroxyprogesterone concentrations. In these cases, testing for CYP11B1 deficiency and 11 beta-hydroxylase 2 (CYP11B2) deficiency should be considered and interpreted as described above. Alternatively, measurement of 21-deoxycortisol might be useful in such cases. This minor pathway metabolite accumulates in CYP21A2 deficiency, as it requires 21-hydroxylation to be converted to cortisol but is usually not elevated in CYP11B1 deficiency since its synthesis requires via 11-hydroxylation of 17-hydroxyprogesterone.

For genetic counseling purposes, identification of asymptomatic carriers of *CYP21A2* variants and deletions is sometimes required. The gold-standard is full DNA sequencing of *CYP21A2*, its pseudogene *CYP21A1P*, and, if possible, recombinants of gene and pseudogene, along with deletion detection. Such a procedure may be costly and complex and often has a slow turnaround time. Therefore, many laboratories perform less complex, but also less complete, variant and deletion assessments, which may miss a significant minority of heterozygote carriers. Biochemical testing using corticotropin (previously adrenocorticotrophic hormone: ACTH) 1-24 adrenal stimulation represents an alternative. However, for 17-hydroxyprogesterone and androstenedione measurements, there is significant overlap between poststimulation results in normal patients and in heterozygote carriers. By contrast, poststimulation 21-deoxycortisol concentrations of 55 ng/dL identify virtually all heterozygote carriers, with minimal overlap with normal individuals.

The goal of congenital adrenal hyperplasia (CAH) treatment is normalization of cortisol levels and, ideally, sex steroid levels. Serum 17-hydroxyprogesterone, androstenedione, and testosterone should be measured and used to guide treatment modifications. Normal prepubertal androgen levels may be difficult to achieve, but if testosterone levels are within the reference range, androstenedione levels up to 100 ng/dL are usually regarded as acceptable. 17-Hydroxyprogesterone levels should not significantly exceed the normal reference range at any time of the day. However, during puberty, the changing levels of sex steroid production may make 17-hydroxyprogesterone measurements less reliable. Since 21-deoxycortisol is not a sex-steroid precursor, its levels appear more reliable during the pubertal period; again, the aim being not to exceed the reference range significantly.

### **Cautions**

At birth, the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-gonadal axis are activated, and all adrenal steroids, including mineralocorticoids and sex steroids and their precursors are high. In preterm infants, the elevations can be even more pronounced due to illness and stress. In doubtful cases, when the initial test was performed on a just-born baby, repeat testing a few days or weeks later is advised.

Corticotropin (previously adrenocorticotrophic hormone: ACTH)1-24 testing has a low but definite risk of drug and allergic reactions and should, therefore, only be performed under the supervision of a physician in an environment that guarantees the patient's safety, typically an endocrine, or other centralized, testing center.

Interpretation of ACTH 1-24 testing in the context of diagnosis of congenital adrenal hyperplasia (CAH) requires considerable experience, particularly for the less common variants of CAH, such as 11-hydroxylase deficiency or

3-beta-hydroxysteroid dehydrogenase (3beta-HSD) deficiency for which very few, if any, reliable normative data exist. For the even rarer enzyme defects, such as deficiencies of StAR (steroidogenic acute regulatory protein), 20,22 desmolase, 17a-hydroxylase/17-lyase, and 17-beta-hydroxysteroid dehydrogenase (17beta-HSD), there are only case reports. Expert opinion from a pediatric endocrinologist with experience in CAH should, therefore, be sought.

**Clinical Reference**

1. Von Schnakenburg K, Bidlingmaier F, Knorr D. 17-hydroxyprogesterone, androstenedione, and testosterone in normal children and in prepubertal patients with congenital adrenal hyperplasia. *Eur J Pediatr.* 1980;133(3):259-267
2. Tonetto-Fernandes V, Lemos-Marini SH, Kuperman H, et al. Serum 21-deoxycortisol, 17-hydroxyprogesterone, and 11-deoxycortisol in classic congenital adrenal hyperplasia: clinical and hormonal correlations and identification of patients with 11beta-hydroxylase deficiency among a large group with alleged 21-hydroxylase deficiency. *J Clin Endocrinol Metab.* 2006;91:2179-2184
3. Idkowiak, J, Cragun, D, Hopkin RJ, and Arlt W. Cytochrome P450 oxidoreductase deficiency. In: Adam MP, Mirzaa GM, Pagon RA, et al, eds. *Gene Reviews* [Internet]. University of Washington, Seattle; 2005. Updated August 3, 2017. Accessed May 2, 2024. Available at [www.ncbi.nlm.nih.gov/sites/books/NBK1419/](http://www.ncbi.nlm.nih.gov/sites/books/NBK1419/)
4. Held PK, Bird IM, Heather NL. Newborn screening for congenital adrenal hyperplasia: review of factors affecting screening accuracy. *Int J Neonatal Screen.* 2020;6(3):67. doi:10.3390/ijns6030067

**Performance****Method Description**

The specimen and an internal standard are assayed by liquid chromatography tandem mass spectrometry. The analyte is detected by multiple-reaction monitoring.(Unpublished Mayo method)

**PDF Report**

No

**Day(s) Performed**

Tuesday

**Report Available**

3 to 10 days

**Specimen Retention Time**

14 days

**Performing Laboratory Location**

Rochester

**Fees & Codes****Fees**

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- Authorized users can sign in to [Test Prices](#) for detailed fee information.
  - Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
  - Prospective clients should contact their account representative. For assistance, contact [Customer Service](#).

**Test Classification**

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

**CPT Code Information**

82542

**LOINC® Information**

Test ID	Test Order Name	Order LOINC® Value
21DOC	21-Deoxycortisol, S	74872-3

Result ID	Test Result Name	Result LOINC® Value
89477	21-Deoxycortisol, S	74872-3